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The Changes in the Growth Pattern of Organs of *Chenopodium rubrum* Photoperiodically Induced to Flowering

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Abstract. The relationship between photoperiodically changed growth of leaves, cotyledons, hypocotyl, roots and flowering has been investigated in *Chenopodium rubrum*. It was found that all the growth characteristics recorded in leaves and cotyledons, *i.e.* length, area, dry weight and chlorophyll content, were inhibited during three inductive photoperiods (16 h darkness, 8 h light — SD) as compared with control plants grown under continuous illumination. Similarly, the cessation of root elongation and a decrease in root dry weight were observed. On the contrary, the elongation and dry weight of hypocotyl are stimulated by SD. The degree of the effect exerted by SD on the growth of different organs depends both on actual growth stage and the number of SD photoperiods. Increased relative rate of growth of roots and cotyledons was recorded in plants transferred after SD treatment to continuous illumination. However, this rise possesses only transitional character and the relative growth rate of treated plants equals that of control ones afterwards. The above growth changes are discussed as a possible modifying factor of floral differentiation.

Additional index words: Growth analysis; photoperiodic induction.

The role of organ correlations in flowering was considered in the past years (Lang 1965) but was believed to be of minor importance. It was only recently that the organ correlations have been recognized as an independent mechanism of flowering control (Miginiac 1978) and its importance stressed in plants with a quantitative character of photoperiodic floral induction (Krekule 1979). On the other hand, it has often been shown that the photoperiodic conditions leading to flowering exert effects on the growth rate and/or initiation of different organs such as leaves (*e.g.* Langer and Bussell 1964) or axillary buds (Thomas 1961, Cook 1976).

We have demonstrated in our previous investigations that the degree of flowering in *Chenopodium rubrum* may be affected by both young organ correlations at the shoot apex (Seidlová and Opatrná 1978) and the character of growth (Teltscherová and Seidlová 1973, Krekule and Seidlová 1973). Thus we found it interesting to investigate how the photoperiodic treatment leading to flowering influences the growth of vegetative organs on the one hand and how this effect may be related to the onset of the reproductive phase on the other. We assume *Chenopodium rubrum* to be an

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advantageous model for such studies. It is characterized by neotenic flowering. Thus, in young and relatively small plants the effect of organ correlations is well noticeable, whereas young, intensively growing tissues are highly responsive to photoperiodic treatment.

This paper summarizes the data we have gained in investigating changes of growth rate of roots, hypocotyl, cotyledons and first leaf induced by short days which bring about flowering. The results concerning the effect of inductive treatment on the differentiation of apical meristem are included.

MATERIAL AND METHODS

Chenopodium rubrum L. (selection 374) was used in our investigations. The seeds were germinated using temperature gradient and the plants were grown in small-volume growth chambers as described earlier (Seidlová and Opatrná 1978). The cultivation was performed on half-strength Knop's solution and under constant conditions (continuous illumination by fluorescent tubes providing illuminance of *ca.* 8000 lx, temperature 20 ± 1 °C). Four or six days old plants were exposed to photoperiodic treatment consisting of one, two or three photoperiods (16 h darkness, 8 h light). The plants were brought back under continuous illumination following the photoperiodic inductions. The growth of the first pair of leaves, cotyledons, hypocotyl and roots similarly as the state of apical meristem were recorded daily.

The following criteria were used to evaluate growth: a) the length of different organs (root, hypocotyl, cotyledon) was measured using slide projector adjusted to ten fold magnification, b) the area of the cotyledons was estimated gravimetrically from a paper-drawn projections of organ shapes, c) the dry weight was estimated after drying the plant material for five hours at 95 °C, d) the chlorophyll content was measured using a Specord spectrophotometer; 80% aceton extract was prepared and absorbance estimated by reading at 662 nm (homogenization of the material was done with sand and solid $MgCO_3$).

The evaluation of data obtained by growth analysis: all the experiments were repeated 3—5 times. The character of the growth curves, including those of timing and degree of response to SD treatment, was similar in all the experiments. The Figures represent average values of all the experiments. Relative growth rate of roots and cotyledons elongation were established from the equation: $R = \frac{\ln a_2 - \ln a_1}{t_2 - t_1} \cdot 100\%$. a_1 denotes the measured value at time t_1, a_2 the value at time t_2.

The initiation and growth of organ primordia at the apical meristem, the initiation and growth of axillary buds at different leaf insertion and the course of differentiation of terminal inflorescence were recorded using a stereomicroscope. Three values were established to investigate apical meristem differentiation: the length of the branching part of the inflorescence, the stage of inflorescence differentiation (vegetative bud — ○, the beginning of differentiation of floral parts of the inflorescence — ◑, terminal flower bud — ●) and the length of terminal flower bud.

RESULTS

The Effect of Three Short Days on Growth and Flowering

The growth of seedling organs of *Chenopodium rubrum* during the first 15 days of ontogenesis under continuous illumination (control plants) is as follows (Fig. 1): the elongation of cotyledons, hypocotyl and roots (Fig. 1-C1, D1) lasts up to sixth day; on seventh day the growth of hypocotyl ceases (Fig. 1-D1); on eigth day the growth of cotyledon's area accelerates (Fig. 1-C3), which positively correlates with the chlorophyll content (Fig. 1-C4) and dry weight (Fig. 1-C2). It is at this moment that the intensive growth of the first leaf pair begins (Fig. 1-B). The rise of root dry weight

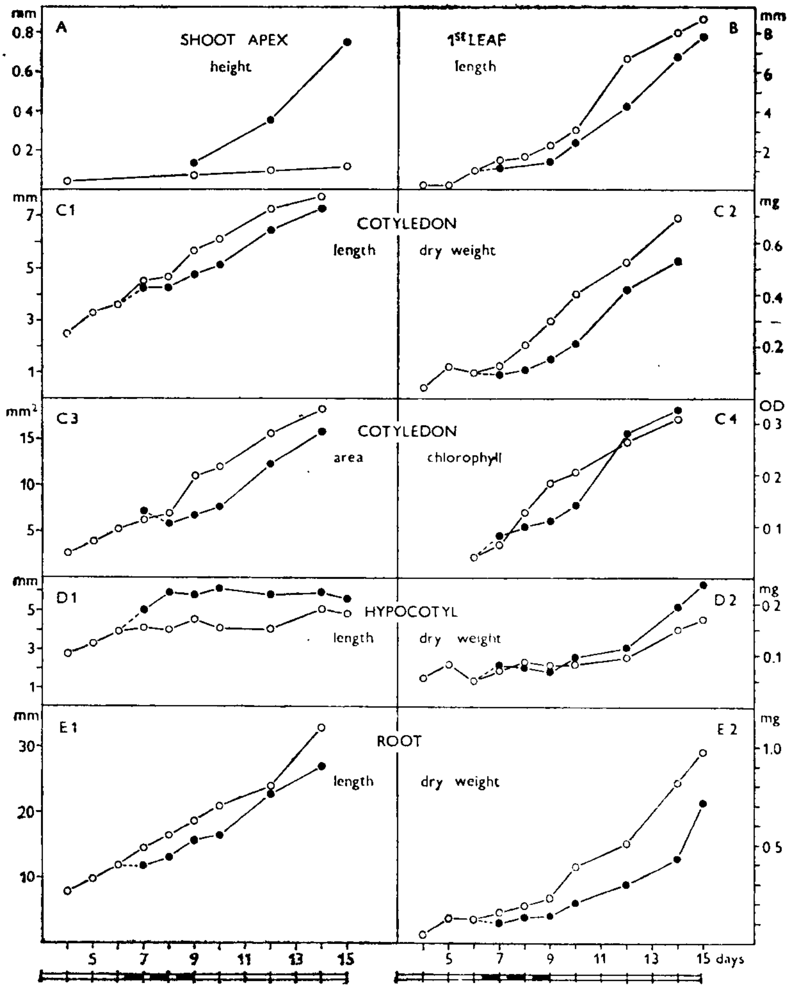

Fig. 1. The effect of three short days (●) on the differentiation of apical meristem (A), growth of the first pair of leaves (B), cotyledons (C), hypocotyl (D) and roots (E) as compared with the growth of non-induced plants grown under continuous illumination (○).

TABLE 1

The comparison of maximal effect of photoperiodic induction consisting of various number of short days on the growth of different organs. The plants were induced at the age of 4 or 6 days and compared with the control (the day of maximal effect is given in the brackets)

The age of plants at the beginning of photoperiodic induction	Six days						Four days	
Number of inductive cycles	■		■ ■		■ ■ ■		■ ■	
Shoot apex								
height [mm]	0.12	(12)	0.30	(12)	0.33	(12)	0.50	(12)
flowering [%]	0	(12)	40	(12)	100	(12)	100	(12)
First leaf								
length [%]	85.9	(12)	74.5	(12)	68.9	(12)	78.9	(12)
Cotyledon								
length [%]	95.6	(9)	87.3	(9)	81.2	(9)	81.4	(9)
dry matter [%]	93.5	(9)	69.2	(9)	60.5	(9)	68.3	(9)
Hypocotyl								
length [%]	112.5	(12)	125.2	(12)	148.7	(12)	130.9	(12)
dry matter [%]	105.5	(12)	112.3	(12)	131.3	(12)	120.0	(12)
Root								
length [%]	98.5	(9)	79.8	(9)	76.6	(9)	78.1	(7)
dry matter [%]	75.0	(9)	70.0	(9)	61.8	(9)	65.9	(9)

on the ninth day (Fig. 1-E2) coincides with the intensive growth of lateral roots. Beginning with the 12th day the elongation of cotyledons slows down (Fig. 1-C1). The dry weight of the hypocotyl (Fig. 1-D2) rises due to vascularization as the vessels from upper nodes enter hypocotyl at the same time. Photoperiodic induction consisting of three short days brings about activation of the youngest axillary buds, formation of inflorescence structure and finally results in the differentiation of terminal flower. All these changes may be simply evaluated using the height of the differentiating terminal bud (Fig. 1-A).

The inhibition of all growth characteristics of leaves (Fig. 1-B), cotyledons (Fig. 1-C) and roots (Fig. 1-E), occurred during induction, as compared with control plants grown under continuous illumination. Such effect is more pronounced in dry weight and with cotyledons also in area growth, rather than in the rate of elongation of a given organ. The relative growth rate rises again after transferring plants to continuous illumination. It reaches either the growth rate of control plants and thus the differences due to the photoperiodic treatment will be retained (see the length of the first leaf pair — Fig. 1-B, the area and dry weight of cotyledons — Fig. 1-C2, C3 and the dry weight of roots — Fig. 1-E2) or is temporarily even higher. The difference between control plants and the treated ones decreases or even disappears in the second case (the length and chlorophyll content in cotyledons — Fig.

1-C1, C4, root length — Fig. 1-E1). The photoperiodic induction also shifts the beginning of intensive area growth in cotyledons (from eight days to ten — Fig. 1-C3) and slows down the lateral root initiation and growth (Fig. 1-E2).

In contrast to all other organs, hypocotyl growth increases due to photoperiodic induction and lasts two days longer as compared with hypocotyl growth under continuous illumination (Fig. 1-D1). The rise in dry weight (Fig. 1-D2) apparently reflects increasing vascularization, with new vessels entering the hypocotyl from differentiating axillary buds.

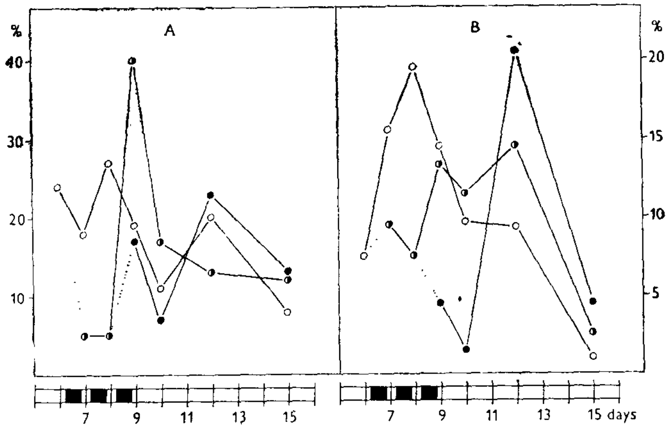

Fig. 2. The comparison of the effect of two (◐) and three (●) short days on the relative growth rate [%] in elongation of roots (A), and cotyledons (B) of six day old plants and of plants grown under continuous illumination (○).

The Effect of One, Two or Three Short Days, on Growth and Flowering

Only three-short-day induction leads to complete (100%) flowering whereas the effect of one or two short days is reflected either in partial flowering and/or in morphological changes of the apical meristem, which represent the early events of transition to reproductive phase (Table 1). We were interested in finding out what are the effects of a single inductive photoperiod and whether the growth effects of three short days may be considered as a summation of single inductive days.

Plants were exposed to one, two or three short days and later cultivated under continuous illumination. The inhibition and/or stimulation due to inductive photoperiods is expressed in terms of percentage of growth of control plants exposed to continuous illumination (Table 1). The results indicate that the degree of growth inhibition of the first leaf pair, cotyledons and roots similarly as the stimulation of hypocotyl reflects the number of inductive short days affecting the plants. Marked differences were found in the way two- or three- day induction brings about following activation of growth and differentiation. Two short days provokes an immediate rise in the relative growth rate in roots (Fig. 2A) and cotyledons (Fig. 2B) under continuous illumination. This activation is 2—3 days delayed in plants treated previously by three short days. It is in this respect that the effect of a single inductive photoperiod cannot be considered as equal.

The Effect of Two or Three Short Days on the Growth and Flowering of Plants Differing in Age

The previous work has demonstrated that younger (four days old) plants are more responsive to induction. The critical number of short days to induce flowering decreases and the differentiation of flowers is more rapid in

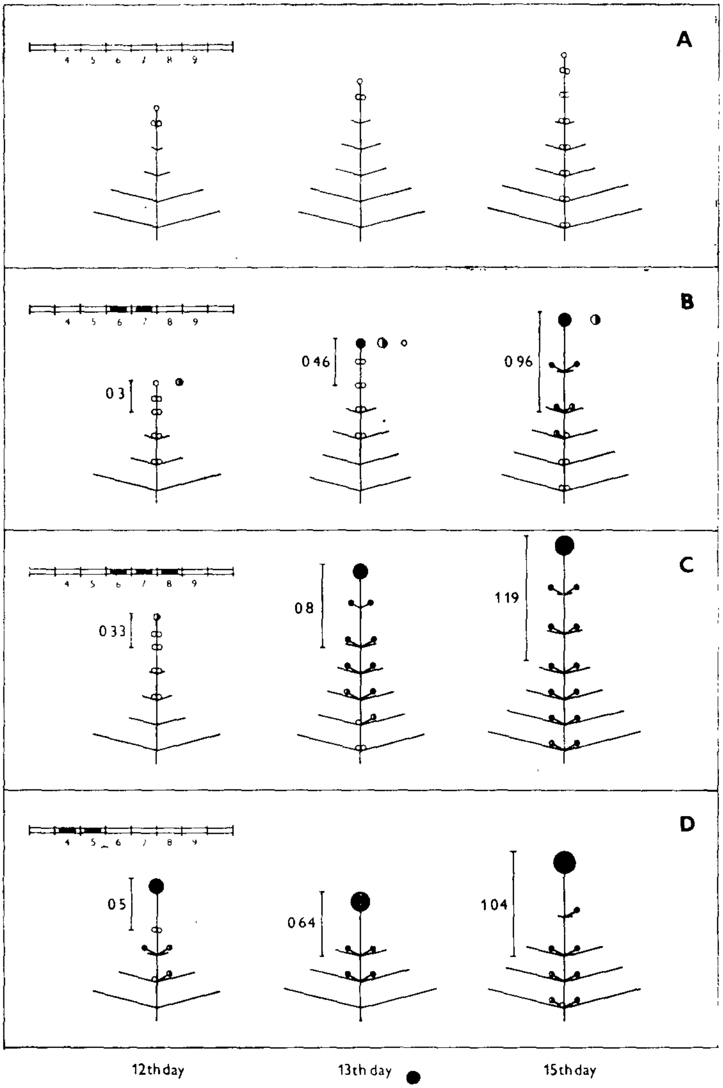

Fig. 3. The comparison of the effect of two and three short days on the differentiation of terminal and axillary buds in plants 4 or 6 days old. (A) plants grown under continuous illumination; (B) plants induced at the age of six days by two short days; (C) plants induced at the age of six days by three short days; (D) plants induced at the age of four days by two short days. The scheme of induction is indicated in each case. ○ — vegetative apical meristem and/or axillary bud; ◐ — the start of differentiation of reproductive organs; ● — fully developed flower bud. The size of the symbol is related to the size of the terminal bud. The number and the accompanying line indicate the size of inflorescence primordia.

young plants (SEIDLOVÁ and KREKULE 1973, KING 1975). Thus we investigated whether there is a differential growth response of six and four day old plants to short days.

The rate of growth and differentiation of axillary buds at different nodes was also evaluated as a new criterion in such investigation (Fig. 3). Three short days bring about the formation of terminal inflorescence and the differentiation of flower buds at all the leaf axils in six day old plants (Fig. 3C). The rate of terminal flower differentiation decreases and there are no flower buds at a lower leaf insertion with only two short days (Fig. 3B). The same induction results in a rapid differentiation of the terminal flower and in flower bud formation in all the leaf axils in plants four days old (Fig. 3D).

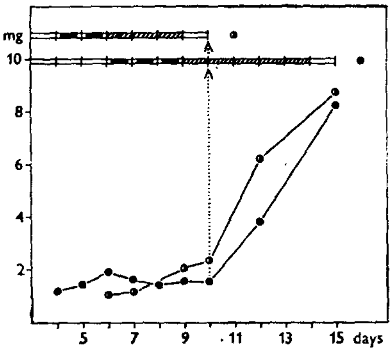

Fig. 4. The growth of lateral roots (↑) accompanied by marked rise in dry weight (on the tenth day) occurs in plants induced at the age of four days by two inductive photoperiods (◐) after flower differentiation, whereas in six day old plants, induced by three inductive photoperiods (●) during flower differentiation (hatched area).

We have found that in contrast to all other organs the growth of the first pair of leaves is less affected by two short days in four day old plants than in six day old (Table 1). As indicated by our previous results (SEIDLOVÁ and OPATRNÁ 1978), the primordium of the first pair of leaves is still a part of terminal inflorescence on the fourth day whereas on the sixth day it is separated from the meristem by primordia of the second and third pairs of leaves. Thus the plant age may markedly affect organ correlations of treated plants. In a similar way the degree of inhibition of cotyledon elongation due to short days is higher at the early stages of its growth. Thus, the effect of two short days in four day old plants is comparable to the effect of three short days in older ones. Such a situation has not been found in dry weight, which may reflect the generally low level of the dry matter accumulation between the fifth and seventh day (Fig. 1).

A high degree of interdependence was established between the plant age and the elongation of hypocotyl due to short day treatment. As found in experiments not quoted here the response falls with plant age. Thus, one inductive period increased the hypocotyl length to 153% in two day old plants, to 129% in three day old plants, to 111% in four day old plants and to 106% in six day old plants, as compared to plants under continuous illumination. The dry matter accumulation of hypocotyl seems to be more affected by the activation of axillary bud growth and is thus rather mediated through the developmental effect of induction.

No differences in the primary root elongation rate were found in relation to plant age (Table 1). We would like to draw attention to the fact that in younger plants (four day old) the differentiation of terminal flower takes place before intensive growth of lateral roots (Fig. 4) expressed as increment in dry weight whereas in older ones both processes coincide.

Thus, in general, the degree of growth effect exerted in vegetative organs of *Chenopodium* by short days depends: on the growth phase of a given organ, on the character of growth and morphogenetic processes and on correlative effects exerted by other organs of the plant.

DISCUSSION

Two classes of effects can be considered when evaluating the results we obtained when affecting the growth of organs by inductive short days: the inhibition (with the exception of hypocotyl which shows stimulation) which is followed by a rise of growth rate. The inhibitory effects of short day have been already reported in our previous work with regard to the anatomical and cytological changes of the apical meristem and to the synthesis of nucleic acids (SEIDLOVÁ 1976, SEIDLOVÁ 1980, OPATRNÁ and HOŘAVKA 1980). Such changes recorded at the apical meristem may be considered as part of evocation. It is not clear if inhibition of vegetative organ growth is similar in nature to the inhibition of apical meristem and whether they are linked. There exist some data in the literature pointing to the growth effects of inductive short days (*e.g.* BÜNNING and KONDER 1954, BÜNSOW 1961, BEEWER and WOOLHOUSE 1975).

The transitional increase in growth rate of vegetative organs as observed after induction in cotyledons, first leaf and in root is compatible with the findings on the increment in internodes and leaves growth and initiation after induction, as reported *e.g.* by SCHWABE (1956) and THOMAS (1961a, b). These phenomena bear some similarity to the activation of apical meristem by induction and possibly rely on similar control. It was along this line that KINET *et al.* (1979) interpreted the rise in mitotic activity in young leaves of *Sinapis* following photoperiodic induction. Considering all the above data and the results of our previous experiments we can describe at least two cases where the character of growth of vegetative organs affects the course of flowering.

Thus, flowering may be linked with root growth, as shown by our previous data on the promotive effect of root removal in flowering (KREKULE and SEIDLOVÁ 1973). An antagonic relationship between inflorescence and root formation as found by DOSTÁL (1950) in *Bryophyllum* should be mentioned in this connection. Similar effects were also convincingly demonstrated by MIGINIAC (1978) using several species. Cytokinin application cancelled flowering enhancement due to root removal. In our experiments the earlier and more rapid differentiation of terminal flower in a younger plant (Fig. 4) was correlated with the delayed growth of lateral roots (it is assumed that branched roots are a richer source of cytokinins). Similar indirect evidence is also provided by the fact that the highest floral effect of root removal (48—72 h after induction), KREKULE and SEIDLOVÁ 1973, coincides (Figs. 1 and 2) with the period of intensive root growth as observed in the above experiments.

The second case represents the relationship between flowering and the character of growth of vegetative organs in postinductive period. A high degree of growth activation was observed in vegetative organs (Fig. 3) and in all parts of terminal bud (Opatrná and Hořavka 1980) following two-day induction. The situation is different with three-day induction, as the inhibition it exhibits is more profound and stretches to postinductive period. The growth activation is thus delayed for one or two days (Fig. 3). These two different conditions are reflected by the rate of terminal flower differentiation, which is either delayed or completely inhibited after two short days and enhanced after three short days. There exists an indirect evidence that such a difference is related not only to the length of inductive period but also to the degree of activation of vegetative growth. The effect of the third inductive day may be substituted by treating the plants either with growth inhibitors (Krekule and Seidlová 1973) or by a short day with light break (Seidlová and Krekule 1973) which *per se* does not possess inductive activity but prevents reappearance of vegetative growth.

Taking into account all the growth changes provoked by inductive photoperiods it seems that only few of them have an impact on flowering. The following paper (Opatrná *et al.* 1980) deals with the possibility of experimentally changing the rate of growth of vegetative organs and establishing their relation to the differentiation of apical meristem.

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BOOK REVIEWS

WATERS, M. D., NESNOW, S., HUISINGH, J. L., SANDHU, S. S., CLAXTON, L. (ed.): APPLICATION OF SHORT-TERM BIOASSAYS IN THE FRACTIONATION AND ANALYSIS OF COMPLEX ENVIRONMENTAL MIXTURES. — Plenum Press, New York—London 1980, 588 pp. US $ 49.50.

The mutagenic and carcinogenic effects of various chemical products, *e.g.* pesticides, drugs and food additives, have been the cause of much public concern and recent scientific study. Their effects may well escape notice until serious and permanent damage has occurred. This evokes the necessity of developing short term bioassays to detect the possible mutagenic effects of all new chemical products and environmental chemicals. This book was published as Volume 15 of the series Environmental Science Research and represents the proceedings of a Symposium convened at Williamsburg, Virginia, U.S.A., February 21—23, 1978. At the symposium 24 contributions were presented which fell under 3 heads. The introductory papers of the proceedings deal with various bioassays used as monitors of environmental mutagens, *e.g.* microbial assay systems (Ames test, DNA repair deficient *E. coli* system, *Saccharomyces cerevisiae* mitotic recombination assay), mammalian cell systems with metabolic activation (Chinese hamster embryo cells, human alveolar macrophages), *in vitro* transformation assays, higher plant monitoring systems (*Tradescantia*, *Osmunda*, *Vicia*) and *Drosophila* screening tests. These papers are followed by a section dealing with the collection and chemical analysis of environmental samples (polluted atmosphere, drinking water). The largest part of the proceedings contains papers on current research into the mutagenic effects of complex organic mixtures. Complex mixtures studied include ambient air and water, drinking water, shale oil, synthetic fuels, automobile exhaust, diesel particulate, coal fly ash, cigarette smoke condensates and food products. All papers are well supported by pertinent and recent references. Readership: all interested in the mutagenic and carcinogenic activities of environmental chemicals.

T. GICHNER (*Praha*)

ROST, T. L., BARBOUR, M. G., THORTON, R. M., WEIER, T. E., STOCKING, C. R.: BOTANY. A BRIEF INTRODUCTION TO PLANT BIOLOGY. — John Wiley & SONS, New York-Santa Barbara-Chichester-Brisbane-Toronto 1979. 344 pp., £ 10.00.

This abridged and extensively revised version of the fifth edition of the identically titled publication by Weier, Stocking and Barbour is one of the prettiest textbooks which has appeared in the field of plant biology. Being supplied with numerous quite original and unusual drawings and photographs, written in a clear style and employing the up-to-date knowledge, it demonstrates how the modern botany book should look like. The book guides the reader through the plant kingdom by giving all important information on cytology, anatomy, morphology, physiology, ecology, taxonomy and evolution of plants. The main topics are logically assorted and attractively discussed and the whole text is well organized pedagogically. For better understanding and orientation of the reader, an extensive glossary is added, which frequently defines the used terms and shows their etymological derivation. The book is provided with a table of metric equivalents, index of plant genera, and a detailed subject index. The excellent graphic and colourful arrangement and the care devoted to the print substantially contribute to the high quality of the book.

DANUŠE HODÁŇOVÁ (*Praha*)